

USSN - 09/896,095

rest of XI - T S S stop--

Please replace the paragraph on page 216, lines 29-31, with the following rewritten paragraph:

--(L) K K S - rest of VIII
ACT.TCC.AG.CTG.AAA.AAG.TCT. (SEQ ID NOs: 98, DNA,
and 99)
rest of XI -T S S stop--

The paragraph on page 26, lines 11-18, has been amended as follows:

Figure 15 shows the main chain of scorpion toxin (Brookhaven Protein Data Bank entry 1SN3) residues 20 through 42 (SEQ ID NO:274). CYS₂₅ and CYS₄₁ are shown forming a disulfide. In the native protein these groups form disulfides to other cysteines, but no main-chain motion is required to bring the gamma sulphurs into acceptable geometry. Residues, other than GLY, are labeled at the β carbon with the one-letter code.

The paragraph on page 110, lines 7-9, has been amended as follows:

5 6 6 7 7
5 0 5 0 3
MVVVIVGATIGIKLFKKFTSKAS (SEQ ID NO:122)

The paragraph on page 151, lines 33-35, has been amended as follows:

10 11 11 12 12 13
5 0 5 0 5 0
EYIGYAWAMVVVIVGATIGIKLFKKFTSKAS (SEQ ID NO:273)

The paragraph on page 154, lines 13-14 14, has been amended as follows:

84 85 86 87 88 89 90 91
GGT|GAT|GAT|CCG|GCC|AAA|GCG|GCC|gcg|cc 3' olig#12 (SEQ
ID NO:123)

USSN - 09/896,095

The paragraph on page 154, lines 23-24, has been amended as follows:

```

      88  89  90  91
GCC|AAA|GCG|GCC|gcg|cc 3' olig#12a (SEQ
ID NO:124)
```

The paragraph on page 154, lines 32-33, has been amended as follows:

```

      88  89  90  91
GCC|AAA|GCG|GCC|gcg|cc 3' olig#12b (SEQ
ID NO:125)
```

The paragraph on page 154, lines 35-36, has been amended as follows:

```

      residue  91  90  89  88  87  86
5' gg|cgc|GGC|CGC|TTT|GGC|CGG|ATC 3'   olig#13 (SEQ ID NO:126)
```

The paragraph on page 203, lines 12-13, has been amended as follows:

Original putative RBS (5'-to-3')

```

GAGCTCagaggCTTACTATGAAAGAAATCTCTGGTTCTTAAGGCTAGC (SEQ ID NO:130)
|SacI|                                     |Nhe I|
```

The paragraph on page 203, lines 16-17, has been amended as follows:

New RBS (5'-to-3')

```

GAGCTCTggaggaAATAAAATGAAAGAAATCTCTGGTTCTTAAGGCTAGC
|SacI|                                     |Nhe I|
(SEQ ID NO:131)
```

The paragraph on page 204, lines 2-6, has been amended as follows:

MB16

```

5'- GATCC tctagagtcggc TTTACA ctttatgcttc (cg-gctcg...-3'
3'-      G agatctcagccg aaatgt gaaatacgaag gc (cgagc...-5'
      |               |-35-               |
      |BamHI           |HpaII|
```

USSN - 09/896,095

(SEQ ID NOs:132 (top), 133 (bottom))

The paragraph on page 204, line 9, has been amended as follows:

MB22 insert

```
5'- GATCC actcccatccccctg TTGACA attaatacat -3'
3'-      G tgaggggtagggggac AACTGT taattagtagc-5'
      |_____|             |_____|             |_____|
      BamHI              -35-              (HpaII)
```

(SEQ ID NOs:134 (top), 135 (bottom))

The paragraph on page 207, lines 28-30, has been amended as follows:

```
.AAA.GCC.CGT.CCG.GAT.-3' (SEQ ID NO:136)
lys ala arg pro asp..... (SEQ ID NO:137)
      |_____|
      AccIII
```

The paragraph on page 208, lines 33-34, has been amended as follows:

```
GCT.CAT.CCG.-3' (SEQ ID NO:138)
ala his pro.... (SEQ ID NO:139)
```

The paragraph on page 222, line 32, has been amended as follows:

5'-gtttcagcggCgCCagaatagaaag-3', (SEQ ID NO:140)

The paragraph on page 224, lines 1-3, has been amended as follows:

```
5'-TATTCTGGCGCCCGT      -3' (SEQ ID NO:141)
3'-ATAAGACCGCGGGCAGGCC-5' (SEQ ID NO:142)
      |_____| |_____|
      NarI   AccIII
```

The paragraph on page 317, line 24-page 318, line 9, has been amended as follows:

One mini-protein motif of potential use comprises a disulfide loop enclosing a helix, a turn, and a return strand.

Such a structure could be designed or it could be obtained from a protein of known 3D structure. Scorpion neurotoxin, variant 3, (ALMA83a, ALMA83b) (hereafter ScorpTx) contains a structure diagrammed in Figure 15 (SEQ ID NO:274) that comprises a helix (residues N22 through N33), a turn (residues 33 through 35), and a return strand (residues 36 through 41). ScorpTx contains disulfides that join residues 12-65, 16-41, 25-46, and 29-48. CYS₂₅ and CYS₄₁ are quite close and could be joined by a disulfide without deranging the main chain. Figure 15 shows CYS₂₅ joined to CYS₄₁. In addition, CYS₃₉ has been changed to GLN. It is expected that a disulfide will form between 25 and 41 and that the helix shown will form; we know that the amino-acid sequence shown (SEQ ID NO:274) is highly compatible with this structure. The presence of GLY₃₅, GLY₃₆, and GLY₃₉ give the turn and extended strand sufficient flexibility to accommodate any changes needed around CYS₄₁ to form the disulfide.

The paragraph on page 334, lines 49-52, has been amended as follows:

k	a	s	.	.	.	(SEQ ID NO:122)
129	130	131	132	133	134	
A.A.r	G.C.n	T.C.n	T.A.r	T.A.r	T.A.r	(SEQ ID NO:143)
	A.G.y	T.G.A	T.G.A	T.G.A		

At the bottom of page 349, as a new paragraph, please add the following:

Homologues 1-19 are SEQ ID NOs:144-162, respectively.

At the bottom of page 351, as a new paragraph, please add the following:

Homologues 20-35 are SEQ ID NOs:163-178, respectively.

At the bottom of page 353, as a new paragraph, please add the following:

Homologues 36-40 are SEQ ID NOs:179-183, respectively.

At page 362, after the line beginning "ALA58", please add the following as a new paragraph:

(SEQ ID NO:144)

The paragraph on page 366, lines 42-48, has been amended as follows:

+ <u>Sfi</u> I	GGCCNNNNNGGCC	P	8	&	5	<N,P,T (SEQ ID NO:184)+
<u>Sma</u> I	CCCGGG	P	3	&	3	<B,M,I,N,P,T
<u>Spe</u> I	ACTAGT	P	1	&	5	<M,N,T
<u>Sph</u> I	GCATGC	P	5	&	1	<B,M,I,N,P,T
<u>Stu</u> I	AGGCCT	P	3	&	3	<M,N,I(<u>Aat</u> I),P,T
% <u>Sty</u> I	CCWWGG	P	1	&	5	<N,P,T
<u>Xca</u> I	GTATAC	P	3	&	3	<N(soon)

On page 368, at the end, as a new paragraph, please add the following:

(SEQ ID NO:185)

On page 369, before the last line, please add the following as a new paragraph:

(SEQ ID NO:185)

The paragraphs on page 374, lines 14-21 (ignoring blank lines) have been amended as follows:

s . . .	(SEQ ID NO:187)	
131 132 133 134		
TCT TAA TAG TGA GGT TAC CAG TCT		502
<u>BstE II</u>		
AAG CCC GCC TAA TGA GCG GGC TTT TTT TTT		532
<u>Trp terminator</u>		
CCT GAG G -3' (SEQ ID NO:186)		539
<u>Sau I</u>		

The paragraphs on page 375, lines 14-21 (ignoring blank lines), have been amended as follows:

```
| s | . | . | . | (residues 128-131 of SEQ ID NO:187)
|131|132|133|134|
|TCT|TAA|TAG|TGA|GGT|TAC|CAG|TCT|
| BstE II |
```

```
|AAG|CCC|GCC|TAA|TGA|GCG|GGC|TTT|TTT|TTT|
| Trp terminator |
```

```
|CCT|GAG|Gca|ggt|gag|cg - 3' (SEQ ID NO:188)
| Sau I | spacer |
```

The paragraph on page 376, lines 15-16 (ignoring blank lines), has been amended as follows:

```
|CCT|GAG|GCA|GGT|GAG|CG (SEQ ID NO:189)
gga ctc cgt cca ctc gc - 5' (SEQ ID NO:190)
```

The paragraph on page 377, last four lines, has been amended as follows:

```
| t | s | k | (SEQ ID NO:192)
|127|128|129|
|ACT|TCG|AAa|gcg|gct|gcg| - 3' (SEQ ID NO:191)
| Asu II | spacer |
```

The paragraph on page 378, lines 14-15 (ignoring blank lines), has been amended as follows:

```
|ACT|TCG|AAA|GCG|GCT|GCG| (SEQ ID NO:193)
tga agc ttt cgc cga cgc - 5' (SEQ ID NO:194)
```

The paragraph on page 379, last four lines, has been amended as follows:

```
| t | s | k | (SEQ ID NO:196)
|127|128|129|
|ttt|acT|TCG|AAa|gcg|tcg|ccg| - 3' (SEQ ID NO:195)
| Asu II |
```

USSN - 09/896,095

The paragraph on page 380, lines 13-14, has been amended as follows:

|TTT|ACT|TCG|AAA|GCG|TCG|CCG| (SEQ ID NO:197)
aaa tga agc ttt cgc agc ggc -5' (SEQ ID NO:198)

The paragraph on page 381, last 4 lines, has been amended as follows:

| k | l | f | k | k | f | t | s | k | (SEQ ID NO:200)
|121|122|123|124|125|126|127|128|129|
|AAA|CTG|TTT|AAG|AAA|TTT|ACT|TCG|AAa|gcg|tcg|ggc| - 3' (SEQ
ID NO:199)

|Asu II| spacer _____|

The paragraph on page 382, lines 12-13 (not counting blank lines), has been amended as follows:

|AAA|CTG|TTT|AAG|AAA|TTT|ACT|TCG|AAA|GCG|TCT|TGA| (SEQ ID NO:201)
 ttt gac aaa ttc ttt aaa tga agc ttt cgc aga act - 5' (SEQ ID NO:202)

The paragraph on page 388, lines 24-29 (not counting blank lines), has been amended as follows:

+
 | s | X | e | d | c | m | (SEQ ID NO:203)
 | 70| 71| 72| 73| 74| 75|
 |TCT|qfk|GAG|GAT|TGC|ATG|C (SEQ ID NO:204) 322
 agc **m ctc cta acg tac gca ccc acc -5' (SEQ ID NO:205)
 | Sph I | spacer |

The paragraph on page 389, last 5 lines, has been amended as follows:

| g | a | (SEQ ID NO:206)
 | 80| 81|
 |GGC|GCC| (SEQ ID NO:207)
 | Bbe I |
 | Nar I |

The paragraph on page 390, lines 23-28 (not counting blank lines) has been amended as follows:

+
 | s | W | X | d | c | m | (SEQ ID NO:208)
 | 70| 71| 72| 73| 74| 75|
 |TCG|TGG|qfk|GAT|TGC|ATG|C (SEQ ID NO:209)
 agc acc **m cta acg tac gcg acc tgc -5' (SEQ ID NO:210)
 | Sph I | spacer |

The paragraph on page 391, last 5 lines, has been amended as follows:

| g | a | (SEQ ID NO:211)
 | 80| 81|
 |GGC|GCC| (SEQ ID NO:212)
 | Bbe I |
 | Nar I |

The paragraph on page 392, lines 21-25 (not counting blank lines), has been amended as follows:

```
| c | r | a | k | (SEQ ID NO:213)
| 61| 62| 63| 64|
|TGC|CGT|GCT|AAG|C (SEQ ID NO:214)
acg gca cga ttc gcg acc ggc (SEQ ID NO:215)
    Esp I spacer
```

The paragraph on page 393, last 5 lines, has been amended as follows:

```
| g | a | (SEQ ID NO:216)
| 80| 81|
|GGC|GCC| (SEQ ID NO:217)
Bbe I
Nar I
```

On page 397, after line 26, please add as a new paragraph:

(SEQ ID NO:218)

On page 398, after line 26, please add as a new paragraph (SEQ ID NO:219)

The paragraph on page 401, lines 16-19 has been amended as follows:

```
| S | . | . | . | (SEQ ID NO:220)
|131|132|133|134|
|TCT|TAA|TGA|TAG| GGTTACC- 486
                   BstE II
```

The paragraph on page 401, lines 26-27, has been amended as follows:

aTCGA (GACctgcaggcatgc)-3' (SEQ ID NO:221)
(SalI) from pGEM polylinker

The paragraph on page 404, lines 16-19, has been amended as follows:

| S | . | . | . | (SEQ ID NO:222)
 |131|132|133|134|
 |TCT|TAA|TGA|TAG| GGTTACC-
BstE II

486

The paragraph on page 404, lines 26-27, has been amended as follows:

aTCGA GACctgca GGTCGACC ggcattgc-3' (SEQ ID NO:223)
SalI

The paragraph on page 410, lines 27-30, has been amended as follows:

| S | . | . | . | (SEQ ID NO:224)
 |131|132|133|134|
 |TCT|TAA|TGA|TAG| GGTTA/CC-
BstE II

The paragraph on page 410, lines 37-38, has been amended as follows:

a/(TCGA),-3' (SEQ ID NO:225)
Sal I

At the bottom of page 413, please add the following new paragraph:

For oligos #801-808 and 810-817, see SEQ ID NOs:226-241 in Table 105.

The paragraph on page 414, lines 3-11 (not counting blank lines) has been amended as follows:

Overlap Sequences	Junction	Tm
AGGCTTACTATGAAG	802:817	42. (SEQ ID NO:242)
TGTCCTTCGCTCG	803:816	42. (SEQ ID NO:243)
CTATTTCTACAATGC	804:815	40. (SEQ ID NO:244)
AACAACCTTTAAATCG	805:814	38. (SEQ ID NO:245)
CCTTCAATTCTCTGC	806:813	44. (SEQ ID NO:246)
CGTTGGTGCTACC	807:812	42. (SEQ ID NO:247)
CCAGTCTAAGCCC	808:811	42. (SEQ ID NO:248)

The paragraph on page 415, lines 1 to end, has been amended as follows:

Table 105: Individual sequences of Oligonucleotides 801-817.

Olig #801 (68 bases)

5'-ggcTTTAcAc TTTATgcTTC cggcTcgTAT AATgTgTggA ATTgTgAgcg cTcAcAATTg AgcTcAgg-3' (SEQ ID NO:226)

Olig #802 (67 bases)

5'-AggcTTAcTA TgAAGAAATc TcTggTTcTT AAggcTAgcg TTgcTgTcgc gAcccTggTA ccTATgT-3' (SEQ ID NO:227)

Olig #803 (70 bases)

5'-TgTccTTcgc TcgTccggAT TTcTgTcTcg AgccAccATA cAcTgggccc TgcAAAgcgc gcATcATccg-3' (SEQ ID NO:228)

Olig #804 (67 bases)

5'-cTATTTcTAc AATgcTAAAg cAggccTgTg ccAgAccTTT gTATAcggTg gTTgccgTgc TAAgcgT-3' (SEQ ID NO:229)

Olig #805 (76 bases)

5'-AAcAAcTTTA AATcggccgA AgATTgcATg cgTAccTgcg gTggcgccgc TgAAggTgAT gATccggccA Aggcg-3' (SEQ ID NO:230)

Olig #806 (67 bases)

5'-ccTTcAATTc TcTgcAAGcT TcTgcTAccg AgTATATTgg TTAcgcgTgg gccATggTgg TggTTAT-3' (SEQ ID NO:231)

Olig #807 (69 bases)

5'-cgTTggTgcT AccATcgggA TcAAAcTgTT cAAGAAgTTT AcTTcgAAgg cgTcTTAATg ATAggTTA-3' (SEQ ID NO:232)

Olig #808 (38 bases)

5'-ccAgTcTAAg cccgccTAAT gAgcgggcTT TTTTTTA-3' (SEQ ID NO:233)

The paragraph on page 416, lines 1 to end, has been amended as follows:

Table 105: Individual sequences of Oligonucleotides 801-817.

(continued)

Olig #810 (29 bases)

5'-TcgATAAAAA AAAAgcccg cTATTAggc-3' (SEQ ID NO:234)

Olig #811 (69 bases)

5'-gggcTTAgAc TggTAAcccT ATcATTAAgA cgccTTcgAA gTAAAcTTcT TgAAcAgTTT gATcccgAT-3' (SEQ ID NO:235)

Olig #812 (65 bases)

5'-ggTAGcAccA AcgATAAccA ccAccATggc ccAcgcgTAA ccAATATAcT cggTAGcAgA AgcTT-3' (SEQ ID NO:236)

Olig #813 (76 bases)

5'-gcAgAgAATT gAAGgccgccc TTggccggAT cATcAccTTc AgcggcgccA

ccgcAggTAc gcATgcAATc
TTcggc-3' (SEQ ID NO:237)

Olig #814 (67 bases)
5'-cgATTTAAAg TTgTTAcgcT TAgcAcggcA AccAccgTAT AcAAAaggTcT
ggcAcAggcc TgcTTTA-3' (SEQ ID NO:238)

Olig #815 (72 bases)
5'-gcATTgTAgA AATAgcggAT gATgcgcgcT TTgcAgggcc cAgTgTATgg
TggcTcgAgA cAgAAATccg Ga-3' (SEQ ID NO:239)

Olig #816 (65 bases)
5'-cgAgcgAAgg AcAAcATAgg TAccAgggTc gcgAcAgcAA cgcTAGccTT
AAGAAccAgA Gattt-3' (SEQ ID NO:240)

Olig #817 (68 bases)
5'-cTTcATAgTA AgccTccTgA gcTcAATTgT gAgcgcTcAc AATTccAcAc
ATTATAcgAg ccggAAgc-3' (SEQ ID NO:241)

The paragraph on page 417, lines 1 to end, has been
amended as follows:

Table 106: Signal Peptides

PhoA	M <u>K</u> q s t i a l a l l p l l f t p v t <u>K</u> A / <u>R</u> T...	(17)
(SEQ ID NO:242)		
MalE	M <u>K</u> I <u>K</u> T G A <u>R</u> i l a l s a l t t m m f s a s a l a / <u>K</u> I...	(18)
(SEQ ID NO:243)		
OmpF	M M <u>K</u> <u>R</u> n i l a v i v p a l l v a g t a n a / a <u>E</u> ...	(19)
(SEQ ID NO:251)		
Bla	M S I Q H F <u>R</u> v a l i p f f a a f c l p v f a / h p...	(>18)
(SEQ ID NO:252)		
LamB	M M I T L <u>R</u> <u>K</u> l p l a v a v a a g v m s a q a m a / v <u>D</u> ...	(19)
(SEQ ID NO:253)		
Lpp	M <u>K</u> A T <u>K</u> l v l g a v i l g s t l l a g / c s...	(>17)
(SEQ ID NO:254)		
gpIII	M <u>K</u> <u>K</u> l l f a i p l v v p f y s h s / a <u>E</u> T V <u>E</u> ...	
(16) (SEQ ID NO:255)		
gpIII-PPTI	M <u>K</u> <u>K</u> l l f a i p l v v p f y s g a / <u>R</u> P <u>D</u> ...	(15)
(SEQ ID NO:256)		
gpVIII	M <u>K</u> <u>K</u> S L V L <u>K</u> a s v a v a t l v p m l s f a / a <u>E</u> G <u>D</u> <u>D</u> ...	
(16) (SEQ ID NO:257)		
gpVIII-BPTI	M <u>K</u> <u>K</u> S L V L <u>K</u> a s v a v a t l v p m l s f a / <u>R</u> P <u>D</u> ...	(15)
(SEQ ID NO:258)		
gpVIII'	M <u>K</u> <u>K</u> s l v l l a s v a v a t l v p m l s f a / a <u>E</u> G <u>D</u> <u>D</u> ...	
(21) (SEQ ID NO:258)		

USSN - 09/896,095

At the bottom of page 420, please add the following new paragraph:

(SEQ ID NO:260)

The paragraph on page 421, lines 10-14, has been amended as follows:

```

                                     / Cleavage site
                                     |
      V   V   P   F   Y   S   H   S   A   E   T   V (SEQ ID
NO:261)
      11  12  13  14  15  16  17  18  19  20  21  22
1609  GTT GTT CCT TTC TAT TCT CAC TCC GCT GAA ACT GTT-3' (SEQ
ID NO:262)
```

The paragraph on page 421, lines 26-30, has been amended as follows:

```

                                     / cleavage site
                                     |
      v   v   p   f   y   s   G   A   a   e   t   v (SEQ ID
NO:263)
      11  12  13  14  15  16  17  18  19  20  21  22
1609  gtt gtt cct ttc tat tct GGc Gcc gct gaa act gtt-3' (SEQ
ID NO:264)
```

The paragraph on page 423, line 18-20, has been amended as follows:

```

      G   A   a   e   t   v   e   s (SEQ ID NO:265)
      77  78  79  80  81  82  83  84
      GGc Gcc gct gaa act gtt GAA AGT
```

At the bottom of page 424, please add the following new paragraph:

(SEQ ID NO:266)

The paragraph on page 427, line 16-19, has been amended as follows:

| S | . | . | . | (SEQ ID NO:268)
 |131|132|133|134|
 |TCT|TAA|TGA|TAG| GGTTACC-
 BstE II

At the bottom of page 427, please add the following new paragraph:

(SEQ ID NO:267)

The paragraph on page 430, lines 24-27, has been amended as follows:

| S | . | . | . | (SEQ ID NO:270)
 |131|132|133|134|
 |TCT|TAA|TGA|TAG| GGTTACC-
 BstE II

The paragraph on page 430, lines 34-35, has been amended as follows:

aTCGA GACctgca GGTTCGAC-3' (SEQ ID NO:269)
 |SalII|

The paragraph on page 433, lines 3-5, has been amended as follows:

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
fM	K	K	L	L	F	A	I	P	L	V	V	P	F	Y
<u>A</u> TG	AAA	AAA	TTA	TTA	TTC	GCA	ATT	CCT	TTA	GTT	GTT	CCT	TTC	TAT
<----- gene III signal peptide ----->														

The paragraph on page 437, lines 13-15, has been amended as follows:

481 482 483 484 485
 N K E S . (SEQ ID NO:272)
 AAT AAG GAG TCT TAA (SEQ ID NO:271)